IN THE SPECIFICATION:

Please amend the specification as shown:

Please delete the paragraph on page 2, lines 24-29 and replace it with the following paragraph:

For example, if cleavage is between lysine and arginine of the amino acid sequence -leucine-tyrosine-lysine-arginine-histidine- (-Leu-Tyr-Lys↓Arg-His-) **(SEQ ID NO: 9)**, leucine is at the P3 position, tyrosine is at the P2 position, lysine is at the P1 position, arginine is at the P1' position, and histidine is at the P2' position.

Please delete the paragraph on page 2, line 36 to page 3, line 10 and replace it with the following paragraph:

OmpT protease cleavage sites have been discovered with amino acid sequences other than basic amino acid pairs, and Dekker et al., using substrates with amino acid substitutions introduced into an OmpT protease substrate comprising the amino acid sequence Ala-Arg-Arg-Ala **(SEQ ID NO: 10)** (P2-P1↓P1'-P2'), have reported that OmpT protease exhibits high specificity for the basic amino acids arginine and lysine as the amino acid at the P1 position of the cleavage site, but is less stringent in regard to the amino acid at the P1' position (Dekker, N. et al. Biochemistry 40: 1694-1701, 2001).

Please delete the paragraph on page 11, line 23 to page 12, line 2 and replace it with the following paragraph:

The discovery that substitution of specific amino acids for the 97th amino acid from the N-terminus of OmpT protease permits actual cleavage of cleavage sites that cannot be cleaved with OmpT protease, as according to aspects (2) and (3) above, is extremely useful as it allows production of target peptides with variety of selection in the types of N-terminal amino acids of

the peptides. In particular, by designing the linker sequence of a fusion protein to be: -Arg-Arg-Arg-Ala-Arg- **(SEQ ID NO: 11)** target peptide, for production of a target peptide using the fusion protein, and utilizing as the processing protease a protease variant having most preferably leucine, methionine or histidine substituting for the 97th aspartic acid from the N-terminus of OmpT protease, it is possible to efficiently and specifically release even polypeptides wherein the N-terminal amino acid is other than lysine or arginine.

Please delete the paragraph on page 14, lines 7-10 and replace it with the following paragraph:

(10) The method of any one of (1) to (9) above, wherein the amino acid sequence from the P5 to P1 positions of the desired cleavage site in the polypeptide or fusion protein is Arg-Arg-Arg-Ala-Arg **(SEQ ID NO: 11)**.

Please delete the paragraph on page 14, lines 11-14 and replace it with the following paragraph:

(11) The method of any one of (1) to (9) above, wherein the amino acid sequence from the P7 to P1 positions of the desired cleavage site in the polypeptide or fusion protein is Asp-Ala-Arg-Arg-Arg-Ala-Arg **(SEQ ID NO: 12)**.

Please delete the paragraph on page 17, lines 13-16 and replace it with the following paragraph:

(25) The method of any one of (12) to (24) above, wherein the amino acid sequence from the P5 to P1 positions of the desired cleavage site in the polypeptide or fusion protein is Arg-Arg-Arg-Ala-Arg **(SEQ ID NO: 11)**.

Please delete the paragraph on page 17, lines 17-20 and replace it with the following paragraph:

(26) The method of any one of (12) to (24) above, wherein the amino acid sequence from the P7 to P1 positions of the desired cleavage site in the polypeptide or fusion protein is Asp-Ala-Arg-Arg-Arg-Ala-Arg **(SEQ ID NO: 12)**.

Please delete the paragraph on page 18, lines 33 to page 19, line 10 and replace it with the following paragraph:

Fig. 1 is a diagram showing the structures of the fusion proteins PRR **(Residues 129-147 of SEQ ID NO: 1)** and PRX. The position of each amino acid is shown on the amino acid sequence of the fusion protein PRR, and the numbers below represent the amino acid sequence numbers from the N-terminus of PRR. β-gal117S4H represents the protecting protein deriving from 117 amino acids from the N-terminus of *E. coli* β-galactosidase, GLP-1(7-37) represents human glucagon-like peptide-1(7-37), and the linker peptide is the portion from amino acid sequence No. 128 (glutamine) to No. 153 (arginine). The OmpT protease cleavage site on the fusion protein PRR is indicated by a black wedge. The fusion protein PRX is a fusion protein wherein arginine at position 141 of PRR is replaced with any of 19 other different amino acids.

Please delete the paragraph on page 19, lines 11-31 and replace it with the following paragraph:

Fig. 2 is a diagram showing the structure of the fusion protein PAn. The position of each amino acid is shown on the amino acid sequence of the fusion protein PA, and the numbers below represent the amino acid sequence numbers from the N-terminus of PA. β-gal117S4H represents the protecting protein deriving from 117 amino acids from the N-terminus of *E. coli* β-galactosidase, GLP-1(7-37) represents human glucagon-like peptide-1(7-37), and the linker peptide is the portion from amino acid sequence No. 128 (glutamine) to No. 153 (arginine). The OmpT protease cleavage site on the fusion protein PA is indicated by a black wedge. The arginine residues introduced by amino acid substitution in the fusion protein PAn are indicated in

bold italics. The OmpT protease cleavage site of PAn is indicated by ↓. At right are shown the cleavage efficiencies for each fusion protein, where the cleavage efficiency of the fusion protein PA is 100%. The letter "a" includes the cleavage efficiency at Arg^{139}-Arg^{140}. The letter "b" includes the cleavage efficiency at Arg^{141}-Arg^{142}. The letter "c" includes the cleavage efficiency at Arg^{143}-Ala^{144}. **Figure discloses residues 129-147 of SEQ ID NO: 2, SEQ ID NOS: 18-27, residues 131-145 of SEQ ID NO: 3 and SEQ ID NOS: 28-29, respectively, in order of appearance.**

Please delete the paragraph on page 19, line 32 to page 20, line 21 and replace it with the following paragraph:

Fig. 3 shows the structures of the fusion proteins PA1A3' **(SEQ ID NO: 30)**, PA1'A3' **(SEQ ID NO: 31)**, PA23' **(Residues 131-145 of SEQ ID NO: 4)**, PA323' **(SEQ ID NO: 32)** and PA2'3' **(SEQ ID NO: 33)**. The position of each amino acid is shown on the amino acid sequence of the fusion protein PA3' **(Residues 129-147 of SEQ ID NO: 3)**, and the numbers below represent the amino acid sequence numbers from the N-terminus of PA3'. β-gal117S4H represents the protecting protein deriving from 117 amino acids from the N-terminus of *E. coli* β-galactosidase, GLP-1(7-37) represents human glucagon-like peptide-1(7-37), and the linker peptide is the portion from amino acid sequence No. 128 (glutamine) to No. 153 (arginine). Arginine residues are shown in bold. The OmpT protease cleavage sites of the fusion protein PA3' are represented by open triangle (cleavage efficiency: 73%) and solid triangle (cleavage efficiency: 220%). The residues introduced by amino acid substitution in PA3' for the fusion proteins PA1A3', PA1'A3', PA23', PA323' and PA2'3' are shown in italics, and the OmpT protease cleavage site is indicated by ↓. At right are shown the cleavage efficiencies for the Arg^{140}-Arg^{141} site (filled circles) and the Arg^{143}-Ala^{144} site (open circles) in each fusion protein, where the cleavage efficiency of the fusion protein PA is 100%. ND stands for "not detected". The letter "a" represents the cleavage efficiency at Arg^{140}-Arg^{141}. The letter "b" the cleavage efficiency at Arg^{139}-Arg^{140}. The letter "c" includes the cleavage efficiency at Arg^{142}-Ala^{143}.

Please delete the paragraph on page 20, line 22 to page 21, line 6 and replace it with the following paragraph:

Fig. 4 shows the structures of the fusion proteins PA3D23' **(SEQ ID NO: 34)**, PA4D23' **(SEQ ID NO: 35)** and PA5D23' **(SEQ ID NO: 36)**. The position of each amino acid is shown on the amino acid sequence of the fusion protein PA23' **(Residues 129-147 of SEQ ID NO: 4)**, and the numbers below represent the amino acid sequence numbers from the N-terminus of PA23'. β-gal117S4H represents the protecting protein deriving from 117 amino acids from the N-terminus of *E. coli* β-galactosidase, GLP-1(7-37) represents human glucagon-like peptide-1(7-37), and the linker peptide is the portion from amino acid sequence No. 128 (glutamine) to No. 153 (arginine). Arginine residues are shown in bold. The main OmpT protease cleavage site of the fusion protein PA23' is represented by a black wedge. The residues introduced by amino acid substitution in PA23' for the fusion proteins PA3D23', PA4D23', PA5D23' and PA23' are shown in italics, and the OmpT protease cleavage site is indicated by ↓. At right are shown the cleavage efficiencies for the Arg^{140}-Arg^{141} site (filled circles) and the Arg^{143}-Ala^{144} site (open circles) in each fusion protein, where the cleavage efficiency of the fusion protein PA is 100%. ND stands for "not detected".

Please delete the paragraph on page 21, lines 7-32 and replace it with the following paragraph:

Fig. 5 shows the structures of the fusion proteins PRMT **(Residues 129-162 of SEQ ID NO: 5)** and PMT **(Residues 129-165 of SEQ ID NO: 6)**. The numbers over the amino acid sequences of the fusion proteins represent the amino acid sequence numbers from the respective N-terminus. β-gal117S4H represents the protecting protein deriving from 117 amino acids from the N-terminus of *E. coli* β-galactosidase, and the linker peptide is the portion from amino acid sequence No. 128 (glutamine) to No. 143 (arginine) in PRMT or from amino acid sequence No. 128 (glutamine to No. 143 (arginine) in PMT. The amino acid sequence up to arginine at position 140 of the fusion protein PRMT matches the amino acid sequence up to arginine at position 140 from the N-terminus of the fusion protein PRR (see Japanese Patent Application No. 2000-602803) whose structure is shown in Fig. 1. Also, the amino acid sequence up to the

arginine at position 143 of the fusion protein PMT matches the amino acid sequence up to arginine at position 143 from the N-terminus of the fusion protein PA23' (Fig. 4). The OmpT protease cleavage site of the fusion protein PMT is indicated by filled circles, and the OmpT protease variant D97M cleavage site is indicated by open circles. RAR-motilin is a polypeptide comprising Arg-Ala-Arg-motilin released from PMT by cleavage at Arg^{140}-Arg^{141}, and RRAR-motilin **(SEQ ID NO: 13)** is a polypeptide comprising Arg-Arg-Ala-Arg-motilin **(SEQ ID NO: 13)** released from PMT by cleavage at Arg^{139}-Arg^{140}.

Please delete the paragraph on page 21, lines 33-36 and replace it with the following paragraph:

Fig. 6 shows the results of HPLC analysis of reactions 25°C, 120 min) between the fusion proteins PRMT and PMT, and the wild-type OmpT protease and OmpT protease variant D97M. **RRAR disclosed as SEQ ID NO: 13.**

Please delete the paragraph on page 22, lines 19-25 and replace it with the following paragraph:

Fig. 9 shows the results of analysis of the reaction solution after 60 minutes by (A) HPLC and (B) SDS-PAGE. Lanes 1: PMT alone; 2: PMT+D97M; 3: motilin sample. Reaction solution composition: 4 M urea, 50 mM sodium phosphate (pH 7.0), 2 mM EDTA, PMT OD_{660} = 50, OmpT D97M OD_{660} = 16; Reaction temperature: 25°C; shaking at 120 min^{-1}. **RRAR disclosed as SEQ ID NO: 13.**

Please delete the paragraph on page 22, line 31 to page 23, line 6 and replace it with the following paragraph:

Fig. 11 shows the structures of the fusion proteins PAC **(Residues 129-167 of SEQ ID NO: 7)** and PCT **(Residues 129-176 of SEQ ID NO: 8)**. The numbers below the amino acids of each fusion protein represent the amino acid sequence numbers from the N-terminus. β-gal117S4H represents the protecting protein deriving from 117 amino acids from the N-terminus

of *E. coli* β-galactosidase, and the linker peptide is the portion from amino acid sequence No. 128 (glutamine) to No. 143 (arginine). The amino acid sequence up to the arginine at position 143 of the fusion protein PMT matches the amino acid sequence up to arginine at position 143 from the N-terminus of the fusion protein PA23' (Fig. 4).

Please delete the paragraph on page 23, lines 7-11 and replace it with the following paragraph:

Fig. 12 shows the results of HPLC analysis for reaction between fusion peptides and wild-type OmpT protease and OmpT protease variants. Reaction was performed at 25°C between PAC and D97L for 10 minutes, and between PCT and D97H for 2 hours. **RRAR disclosed as SEQ ID NO: 13.**

Please delete the paragraph on page 23, lines 15-27 and replace it with the following paragraph:

Fig. 14 shows the SDS-PAGE analysis results indicating release of human motilin from the fusion protein PMT using inclusion bodies obtained from W3110 M25-transformed *E. coli* co-expressing the fusion protein PMT and the OmpT protease variant D97M, prepared for Example 17. Mr = protein molecular weight markers; Lanes 1: 20 minutes, 2: 40 minutes, 3: 60 minutes, 4: 120 minutes, 5: 180 minutes, 6: 240 minutes, 7: 300 minutes, 8: 360 minutes, 9: 1440 minutes (24 hrs) after start of reaction, 10: motilin sample. Reaction mixture composition: 4 M urea, 50 mM sodium phosphate (pH 7.0), 2 mM EDTA, inclusion body OD_{660} = 20, Reaction temperature: 25°C. **RRAR disclosed as SEQ ID NO: 13.**

Please delete the paragraph on page 23, line 28 to page 24, line 9 and replace it with the following paragraph:

Fig. 15 shows the structures of the fusion proteins PMT **(Residues 129-165 of SEQ ID NO: 6)**, PMT6D **(SEQ ID NO: 37)** and PMT7D **(SEQ ID NO: 38)**. The numbers over the amino acid sequences of the fusion proteins represent the amino acid sequence numbers from the

respective N-terminus. β-gal117S4H represents the protecting protein deriving from 117 amino acids from the N-terminus of *E. coli* β-galactosidase, and the linker peptide is the portion from amino acid sequence No. 128 (glutamine) to No. 143 (arginine). The amino acid sequence up to arginine at position 143 in the fusion proteins matches the amino acid sequence up to arginine at position 143 from the N-terminus of the fusion proteins PA23', PA3D23' and PA4D23' (Fig. 4). The cleavage sites of the fusion proteins by the OmpT protease variant D97M are indicated by arrows. AR-motilin is a polypeptide comprising Ala-Arg-motilin released by cleavage at Arg^{141}-Ala^{142}, and RRAR-motilin **(SEQ ID NO: 13)** is a polypeptide comprising Arg-Arg-Ala-Arg-motilin **(SEQ ID NO: 13)** released by cleavage at Arg^{139}-Arg^{140}.

Please delete the paragraph on page 24, lines 10-15 and replace it with the following paragraph:

Fig. 16 shows the results of HPLC analysis for reaction (25°C, 120 min) between the fusion protein PMT and the OmpT protease variant D97M. The numbers in parentheses indicate each by-product concentration, where the concentration of motilin produced from the fusion protein is 100. **RRAR disclosed as SEQ ID NO: 13.**

Please delete the paragraph on page 24, lines 16-21 and replace it with the following paragraph:

Fig. 17 shows the results of HPLC analysis for reaction (25°C, 120 min) between the fusion protein PMT6D and the OmpT protease variant D97M. The numbers in parentheses indicate each by-product concentration, where the concentration of motilin produced from the fusion protein is 100. **RRAR disclosed as SEQ ID NO: 13.**

Please delete the paragraph on page 26, lines 2-13 and replace it with the following paragraph:

For fusion protein PA3' wherein arginine is present at position P3' (cleavage site adjacent amino acid sequence = -Ala-Ala-Arg[P1]-Arg[P1']-Ala-Arg[P3']-Ala[P4']-Ala-) **(SEQ ID NO:**

14), it was found that cleavage also occurs between arginine at position P3' and alanine at position P4', and a sequence was discovered that allowed efficient cleavage at the arginine-alanine site. Since the fact that the substrate was efficiently cleaved with a sequence other than one having consecutive basic amino acids is extremely important for using OmpT protease as a processing enzyme, the present inventors carried out further investigation.

Please delete the paragraph on page 26, lines 14-24 and replace it with the following paragraph:

Upon investigating various amino acid sequences based on the knowledge that cleavage efficiency is increased by situating arginine at the cleavage site adjacency and the knowledge that three consecutive arginines render cleavage between arginine-arginine more difficult, it was found that in the amino acid sequence -Arg-Arg-Arg-Ala-Arg-Ala- **(SEQ ID NO: 15)**, the major cleavage occurs at -Arg-Arg-Arg-Ala-Arg↓Ala- **(SEQ ID NO: 15)**. In other words, this demonstrated a property whereby situating three consecutive basic amino acids promotes cleavage at basic amino acid sites thereafter.

Please delete the paragraph on page 26, line 25 to page 27, line 6 and replace it with the following paragraph:

However, cleavage did occur in the aforementioned amino acid sequence (-Arg-Arg-Arg-Ala-Arg-Ala-) **(SEQ ID NO: 15)** even in the three consecutive arginine residue sequence. In order to inhibit this, the amino acid sequence-Asp-Ala-Arg-Arg-Arg-Ala-Arg↓Ala- **(SEQ ID NO: 16)** was constructed having aspartic acid situated as an acidic amino acid in the amino acid sequence upstream from the N-terminal end. The arginine-alanine cleavage efficiency was reduced by half using this sequence, but cleavage in the three consecutive arginine sequence was successfully inhibited. That is, cleavage by OmpT protease may be optimized for easier cleavage between arginine-alanine in -Arg-Arg-Arg-Ala-Arg↓Ala- **(SEQ ID NO: 15)** and Asp-Ala-Arg-Arg-Arg-Ala-Arg↓Ala- **(SEQ ID NO: 16)**. It was thought that using these sequences (-Arg-Arg-Arg-Ala-Arg-Ala- **(SEQ ID NO: 15)** and Asp-Ala-Arg-Arg-Arg-Ala-Arg-Ala- **(SEQ**

ID NO: 16)), and most preferably Asp-Ala-Arg-Arg-Arg-Ala-Arg-Ala- **(SEQ ID NO: 16)**, would allow efficient cleavage even when the P1' position is an amino acid other than alanine.

Please delete the paragraph on page 27, lines 7-16 and replace it with the following paragraph:

Based on these results, motilin (with phenylalanine as the N-terminal amino acid) was examined as a target polypeptide to determine whether or not situating a physiologically active peptide in the amino acid sequence at the C-terminal end of the cleavage site -Arg-Arg-Arg-Ala-Arg↓Ala- **(SEQ ID NO: 15)** permits direct cleavage of the physiologically active peptide from a fusion protein with OmpT protease. Fusion protein PMT was constructed with motilin as the target polypeptide, and was reacted with OmpT protease in an attempt to cut off motilin.

Please delete the paragraph on page 30, lines 14-24 and replace it with the following paragraph:

The HPLC analysis was carried out using a YMC PROTEIN RP column, with a column temperature of 40°C and a flow rate of 1 mL/min. After rinsing with 10% acetonitrile containing 0.1% trifluoroacetic acid for 3 minutes, elution was performed with a linear gradient of 10-15% acetonitrile containing 0.1% trifluoroacetic acid for 10 minutes. Absorption at 220 nm was monitored, and the decomposition product peptide Tyr-Gly-Gly-Phe-Leu-Arg **(SEQ ID NO: 17)** was detected. The OmpT protease activity upon cleavage of 1 μmol dynorphin A at 25°C for 1 minute was defined as 1 unit.

Please delete the paragraph on page 40, lines 15-23 and replace it with the following paragraph:

This suggests the possibility that when OmpT protease is used to cut off a target polypeptide, wherein the N-terminal amino acid is any of the 17 amino acids other than aspartic acid, glutamine and proline, from a fusion protein having the structure: protecting protein-linker

peptide-target polypeptide, specific cleavage is possible by situating the target polypeptide after the C-terminal of the amino acid sequence -Asp-Ala-Arg-Arg-Arg-Ala-Arg- **(SEQ ID NO: 12)**.

Please delete the paragraph on page 41, line 35 to page 42, line 9 and replace it with the following paragraph:

This demonstrated that human motilin cannot be cut off at the primary peptide cleavage site by OmpT protease, simply by using -Arg-Arg-Arg-Ala-Arg-motilin **(SEQ ID NO: 11)** as the amino acid sequence adjacent to the cleavage site. It was thus suggested that, while the substrate specificity of this protease is tolerant with regard to the amino acid at position P1', more efficient cleavage requires introduction of a mutation into the protease itself, to increase the specificity for the amino acid at position P1'. Thus, an OmpT protease variant was created and it was examined whether or not primarily human motilin can be cut off from the fusion protein using it.

Please delete the Table 2 header on page 49 and replace it with the following header:

Table 2 Release of motilin from fusion protein PMT by OmpT protease variant OmpT D97M **(RRAR disclosed as SEQ ID NO: 13)**

Please delete the paragraph on page 53, lines 19-30 and replace it with the following paragraph:

There was detected not only human motilin produced by cleavage at Arg^{143}-Phe^{144}, but also a polypeptide (RRAR-motilin) **(SEQ ID NO: 13)** produced by cleavage at Arg^{139}-Arg^{140}. In SDS-PAGE, RRAR-motilin **(SEQ ID NO: 13)** was seen with a more concentrated band than human motilin (Fig. 9B), but the HPLC analysis results contradicted this, indicating a greater area for the peak of human motilin than RRAR-motilin **(SEQ ID NO: 13)**, and thus a larger amount (Fig. 9A). This was assumed to be because RRAR-motilin **(SEQ ID NO: 13)** is more easily dyeable than human motilin in SDS-PAGE. Thus, it is believed that the band densities are correct as the results of SDS-PAGE and do not reflect the volume ratios.

Please delete the paragraph on page 55, line 34 to page 56, line 11 and replace it with the following paragraph:

No by-products were released by cleavage at other sites. The fusion protein PCT was cleaved at Arg^{139}-Arg^{140} and Arg^{140}-Arg^{141} by wild-type OmpT protease, releasing RRAR-CT **(SEQ ID NO: 13)** and RAR-CT. PCT was cleaved at Arg^{139}-Arg^{140}, Arg^{141}-Ala^{142} and Arg^{143}-Cys^{144} by D97H, releasing RRAR-CT **(SEQ ID NO: 13)**, AR-CT and human calcitonin precursor. Release of the target physiologically active polypeptide by wild-type OmpT protease was confirmed from all of the fusion proteins. This demonstrated that physiologically active polypeptide production systems utilizing linker polypeptide sequences and OmpT protease variants indicated in the examples can be applied not only for specific physiologically active polypeptides, and therefore the general utility of this method is thought to be considerable.

Please delete the paragraph on page 57, line 31 to page 58, line 2 and replace it with the following paragraph:

While the results of Example 14 demonstrated that motilin is produced from the fusion protein PMT by the OmpT protease variant D97M, cleavage also occurred at Arg^{139}-Arg^{140} to yield the by-product RRAR-motilin **(SEQ ID NO: 13)**. On the other hand, the results of Example 8 indicated that situating the acidic amino acid aspartic acid at position P3 or P4 where cleavage is not desired can inhibit cleavage at those sites.

Please delete the paragraph on page 58, line 35 to page 59, line 15 and replace it with the following paragraph:

Figs. 16-18 also show the concentrations of the by-products AR-motilin (produced by cleavage at Arg^{141}-Ala^{142}) and RRAR-motilin) **(SEQ ID NO: 13)** produced by cleavage at Arg^{139}-Arg^{140}), where the concentration of motilin released from each is defined as 100. By-products were yielded at 2.8 and 33% with PMT (Fig. 16) and at 3.5 and 16% with PMT6D (Fig. 17), and therefore RRAR-motilin **(SEQ ID NO: 13)** production was particularly inhibited. Also, no peak for RRAR-motilin **(SEQ ID NO: 13)** was detected with PMT7D (Fig. 18). This matches with the results of Example 8, demonstrating that even when using OmpT variant

enzymes, situating the acidic amino acid aspartic acid at the P3 or P4 position, at which cleavage is not desired, can inhibit cleavage at those sites. Motilin was released in the greatest amount from PMT7D (370 μg/mL), but this was ascribed to an increased motilin release concentration permitted by the lack of by-product.